

## PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2001-157574

(43)Date of publication of application : 12.06.2001

(51)Int.Cl. C12M 3/00  
C08J 9/00  
C12N 5/06  
// C08L101/16

(21)Application number : 11-340568

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(22)Date of filing : 30.11.1999

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(54) HONEYCOMB STRUCTURE, METHOD FOR PREPARING THE STRUCTURE, FILM AND CELL CULTURE BASE USING THE STRUCTURE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a step when a base and/or a cell forms a three dimensional structure in culturing a cell.

SOLUTION: A hydrophobic organic solvent solution of a polymer comprising 50-99% of a biodegradable polymer (polylactic acid) and 50-1% of amphiphatic polymer (Cap) is cast to a base at 50-95% relative humidity under atmospheric pressure. The organic solvent is gradually transpired and simultaneously condensed on the surface of the cast solution and minute water drops formed by the condensation are evaporated to give a honeycomb structure. The sheet or the cell culture base is obtained by using this honeycomb structure.



図1. ポリ乳酸: Cap. a=1, b=4:1, a-b=1とした時のハニカム構造体の光学顕微鏡写真

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## LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

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CLAIMS

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[Claim(s)]

[Claim 1] The honeycomb structure object with which a biodegradability polymer is obtained because 50-99 w/w% and an amphiphilic polymer evaporate the minute waterdrop which was made to dew on this cast liquid front face while carrying out the cast of the hydrophobic organic solvent solution of the polymer which consists of 50-1 w/w% on a substrate under atmospheric air of 50 - 95% of relative humidity and transpiring this organic solvent gradually, and was produced by this dew condensation.

[Claim 2] The film which consists of a honeycomb structure object of claim 1.

[Claim 3] The honeycomb structure object of claim 1 said whose biodegradability polymer is aliphatic series polyester.

[Claim 4] The base material for cell cultures which consists of a honeycomb structure object according to claim 1.

[Claim 5] The base material for cell cultures according to claim 4 whose diameter of said honeycomb structure object is 0.1-10 micrometers.

[Claim 6] How to prepare a honeycomb structure object by making it dew on this cast liquid front face at the same time it carries out the cast of the hydrophobic organic solvent which carries out 1-50% content of 50-99% and the amphiphilic polymer for a biodegradability polymer under relative humidity 50-95% of atmospheric air on a substrate and transpires this organic solvent gradually.

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DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Field of the Invention] This inventions are the foundation of the cell culture from which research serves as a foundation most in a prosperous cell technology and systems engineering in recent years in medicine or the agricultural field, and an object in connection with application. That is, a scaffold in case the base material which is in charge of cultivating a cell, and/or a cell form a three-dimension organism is offered. [0002]

[Description of the Prior Art] In the interaction of a cell and an ingredient, it is known that a cell will be influenced not only with the chemical property on the front face of an ingredient but with a detailed configuration. Then, when aiming at functional control of a cell from viewpoints, such as systems engineering, processing of the chemical property on the front face of an ingredient in contact with a cell and the both sides of detailed structure becomes important. Installation of the size control of the cell adhesion side using the micro pattern technique used for semiconductor industry etc. as a surface treatment technique as a surface micro-processing method and the minute slot structure to a substratum and production of the detailed irregularity by the microsphere are performed, and it is known that the surface fine structure will affect growth of a cell etc. greatly. [0003] A very advanced technique is required for a surface setup using these micro pattern techniques, and the present condition is having many problems, such as mass production method being impossible and becoming high cost. It is known that the film which has the honeycomb structure of mum scale by carrying out the cast of the dilute solution of the polymer which has structure completely special as another surface patterning technique under high humidity will be obtained. It is the description that this approach is excellent in the economical efficiency which hits to carry out patterning. [0004] the rod-coil which will specifically become 283 volumes and a page 373 from a hydrophilic block and a hydrophobic block Science and 1999 -- a jib -- the polyphenyl quinoline-block-polystyrene which is a lock polymer -- using -- \*\*\*\* -- moreover, the jib which will become 369 volumes and a page 387 from polystyrene and the poly para-phenylene which is an upright block Nature and 1994 -- the example using a lock polymer is indicated. Thus, at the Prior art, the special polymer having a part with the strong autoagglutination force and the part which discovers flexibility was used, these polymers were dissolved in the hydrophobic organic solvent, and the honeycomb structure object was prepared by carrying out the cast of this. On the other hand, this invention persons are SHINSO lids. Films The 1998 327 to 329 volumes, A page 854, Supra molecular Science 1998 The 5th volume, a page 331 -- and molecular Crystal Liquid Crystal In 1998 The 322nd volume The acrylamide polymer of a hydrophilic property is made into a principal chain frame at a page 305. The amphiphilic polymer which has a lactose radical or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or it has reported giving the thin film which has honeycomb structure by the approach with the same ion complex of anionic polysaccharides, such as heparin and dextran sulfate, and the long-chain alkyl ammonium salt of the 4th class. [0005] However, since it was inferior to the self-independence nature of the honeycomb structure object acquired in these polymers or had the fault of honeycomb structure collapsing with time, it was not what offers function sufficient as a base material for cell cultures. [0006]

[Problem(s) to be Solved by the Invention] When performing a cell culture in a cell technology, systems engineering, etc., the base material used as the scaffold of a cell is required, and it is known in the interaction of a cell and an ingredient like the above-mentioned that a cell will be influenced not only with the chemical property on the front face of best but with a detailed configuration. When aiming at functional control of a cell, a design of the chemical property on the front face of an ingredient and the both sides of structure with a detailed cell in contact with a cell becomes important. With the porous film which has honeycomb structure, a honeycomb pattern offers a cell adhesion side and it is shown that a vesicular structure serves as access to the support substrate of a cell and the supply root of a nutrition. [0007] If a cell is systematized based on this honeycomb structure film, an artificial organ can be considered as that one usage. However, since embedding inside of the body becomes indispensable when it is made an artificial organ etc., as for this base material, being absorbed to the living body is desirable in the long run. The time amount which a cell culture takes with the ingredient which gives old honeycomb structure maintains structure to stability, and there is nothing that was made from a biodegradability ingredient which is disassembled more than at it. In other words, in combining a honeycomb structure object, a cell technology, and a cell culture technique, and developing to medical-application ways, such as an artificial organ, it is indispensable to use a biodegradability ingredient. [0008]

[Means for Solving the Problem] As a result of inquiring wholeheartedly in consideration of an above-mentioned technical problem and a trouble, this invention person is combining a biodegradable polymer and an amphiphilic polymer at a suitable rate, economical preparation is possible for him, he has independence nature, and it found out giving a also structurally stable honeycomb structure object. That is, this invention is attained by the following. [0009] (1) The honeycomb structure object with which a biodegradability polymer is obtained because 50-99 w/w% and an amphiphilic polymer evaporate the minute waterdrop which was made to dew on this cast liquid front face while carrying out the cast of the hydrophobic organic solvent solution of the polymer which consists of 50-1 w/w% on a substrate under atmospheric air of 50 - 95% of relative humidity and transpiring this organic solvent gradually, and was produced by this dew condensation.

The film which consists of a honeycomb structure object of (2) and (1).

(3) The honeycomb structure object of (3) said whose biodegradability polymer is aliphatic series polyester.

The base material for cell cultures which consists of a honeycomb structure object of (4) and (1).

(5) The base material for cell cultures according to claim 4 whose diameter of said honeycomb structure object is 0.1-10 micrometers.

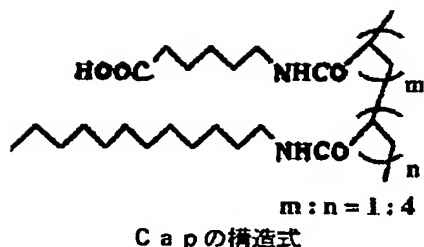
(6) How to prepare a honeycomb structure object by making it dew on this cast liquid front face at the same time it carries out the cast of the hydrophobic organic solvent which carries out 1-50% content of 50-99% and the amphiphilic polymer for a biodegradability polymer under relative humidity 50-95% of atmospheric air on a substrate and transpires this organic solvent gradually. [0010]

[Embodiment of the Invention] As a biodegradability polymer in this invention, it is polylactic acid. Aliphatic series polycarbonates, such as biodegradability aliphatic series polyester, such as polyhydroxy butanoic acid, the poly caprolactone, a polyethylene horse mackerel peat, and a polybutylene horse mackerel peat, polybutylene carbonate, and polyethylene carbonate, etc. are desirable from a soluble viewpoint to an organic solvent. Especially, polylactic acid and the poly caprolactone are desirable from viewpoints, such as an ease of acquisition, and a price. [0011] From it being indispensable that there is no toxicity if it takes into consideration to use as a cell culture base material as an amphiphilic polymer used for this invention A polyethylene glycol / polypropylene-glycol block copolymer, The amphiphilic polymer which makes an acrylamide polymer a principal chain frame and has a lactose radical or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or the ion complex of anionic macromolecules, such as heparin, dextran sulfate, and DNA, a nucleic acid of RNA, and long-chain alkyl ammonium salt, It is desirable to use the amphiphilic polymer which made the

hydrophilic radical water-soluble protein, such as gelatin, a collagen, and albumin. [0012] In producing the honeycomb structure object of this invention, it is required to be nonaqueous solubility as an organic solvent used from it being indispensable to make a minute waterdrop particle form on a polymer solution. As these examples, nonaqueous solubility ketones, such as ester, such as aromatic hydrocarbon, such as halogen system organic solvents, such as chloroform and a methylene chloride, benzene, toluene, and a xylene, ethyl acetate, and butyl acetate, and methyl isobutyl ketone, a carbon disulfide, etc. are mentioned. Whether it uses it independently or uses these organic solvents as a mixed solvent which combined these solvents, they are not cared about. the polymer concentration of a biodegradability polymer and amphiphilic polymer both \*\*\*\*\* dissolved in these -- 0.01 to 10wt(s)% -- it is 0.05 to 5wt(s)% more preferably. The dynamics reinforcement of the film which will be obtained if polymer concentration is lower than 0.01wt(s)% runs short and is not desirable. Moreover, more than at 10wt%, polymer concentration becomes high too much and sufficient honeycomb structure is not acquired. Moreover, the presentation ratio of a biodegradability polymer and an amphiphilic polymer is 99:1 to 50:50 (wt/wt). honeycomb structure with an amphiphilic polymer ratio uniform at one or less obtains -- not having -- moreover -- this -- it is not desirable in order to apply to the stability of the honeycomb structure object with which a ratio is obtained or more by 50, and dynamic stability especially. [0013] Although the cast of this polymer organic solvent solution is carried out on a substrate in this invention and a honeycomb structure object is prepared, liquids, such as a macromolecule which was excellent in products made from an organic solvent-proof, such as inorganic materials, such as glass, a metal, and a silicon wafer, polypropylene, polyethylene, and a polyether ketone, as this substrate, water, a liquid paraffin, and a liquefied polyether, can be used. Especially, when water is used for a base material, this structure can be independently taken out from a substrate easily by employing efficiently the independence nature which is the description of this honeycomb structure object, and it is suitable. [0014] The device in which honeycomb structure is formed by this invention is considered as follows. When a hydrophobic organic solvent evaporates, in order to take the latent heat, the temperature of a cast philharmonic front face falls, and the drop of minute water condenses and adheres to a polymer solution front face. work of the hydrophilic part in a polymer solution -- the surface tension between water and a hydrophobic organic solvent -- decreasing -- this sake -- a water particle -- condensing -- one lump -- it is going to become -- the time -- carrying out -- stabilizing -- having . A solvent follows on evaporating, and it stands in a line in the form in which the drop which carried out the hexagonal form carried out the closest packing, and finally, water flies and it remains as a form where the polymer was regularly located in a line in the shape of a honeycomb. Therefore, it is desirable for relative humidity to be in 50 to 95% of range as an environment where this film is prepared. At 50% or less, dew condensation of a up to [ a cast film ] becomes inadequate, and it is not [ at 95% or more, environmental control is difficult, and ] desirable. Thus, the magnitude of each of the made honeycomb structure objects (each) is 0.1 to 10 micrometers, and if it is the magnitude of this range, it can be suitably used as a base material for cell cultures.

[0015] Hereafter, although this invention is explained to a detail using an example, this invention is not limited at all by this. [0016]

[Example] (Example 1-3) The chloroform solution (1.0g/L) of Polly L-lactic acid (molecular weight 85000-160000) and the benzene solution (1.0g/L) of Cap of a formula (I) were mixed at a rate of 1:1, 4:1, and 8:1, and the cast was carried out on the glass substrate, it put gently on the bottom of a room temperature and the condition of 80% of humidity, and the honeycomb structure object was prepared by flying a solvent gradually. In this way, the optical microscope photograph of the obtained structure is shown in drawing 1 . These films can be picked up with pincettes and it was checked that free-standing is shown. [Formula 1]



[0017] (Example 4) Milli-Q water (40ml) was put into the petri dish (bore of 9.3cm), the chloroform solution (1.0 g/L) of Polly L-lactic acid (molecular weight 85000-160000) and the benzene solution (1.0 g/L) of Cap which is an amphiphilic polymer were mixed at a rate of 8:1 (wt%), the 20microl was developed on the water surface, and the collapse film was produced. Then, this polymer solution of 10 moremicrol was dropped, the drop was made to form, and the honeycomb structure object was produced by applying the air of 80% of relative humidity at the rate of 90 ml/min to it. This structure could be dipped up on the frame ( $\phi=5\text{mm}$ ), and has checked free-standing. [0018] (Example 1 of a comparison) Only with the chloroform solution (1.0 g/L) of Polly L-lactic acid (molecular weight 85000-160000), adjustment of honeycomb structure was tried by the same actuation as an example 1. Although the result was shown in drawing 2, the mol follow G of the obtained film was heterogeneous.

[0019] (Example 2 of a comparison) Preparation of a honeycomb structure object was tried on an example 1 and coincidence conditions only using the Cap solution. The film was beaten by this example while minute waterdrop evaporated, and by it, it did not have free-standing. [0020] (Example 1 of a trial) The honeycomb film obtained in the example 1 was installed on the glass plate which carried out the Pori HEMA coat, and the cow main artery origin vascular endothelial cell (ECs) was cultivated on this. Culture was performed using the IMDM culture medium within the CO2 incubator (CO2 concentration =5%, temperature =37 degree C, relative humidity = 80%). Seeding of the ECs was directly carried out on the glass plate of a pHEMA coat as an example of a comparison, and it cultivated on the same conditions. In the former, it turned out that it pasted up well, the cell is extended and the film of a honeycomb structure object is functioning as a scaffold of a cell. On the other hand, in the latter, ECs was not pasted up at all. [0021] [Effect] As mentioned above, according to the approach of this invention, preparation of the honeycomb structure object which used the biodegradability polymer as the principal component and which was arranged regularly is attained simple, and offer of the film and cell culture base material using this is attained.

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DRAWINGS

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[Drawing 1]

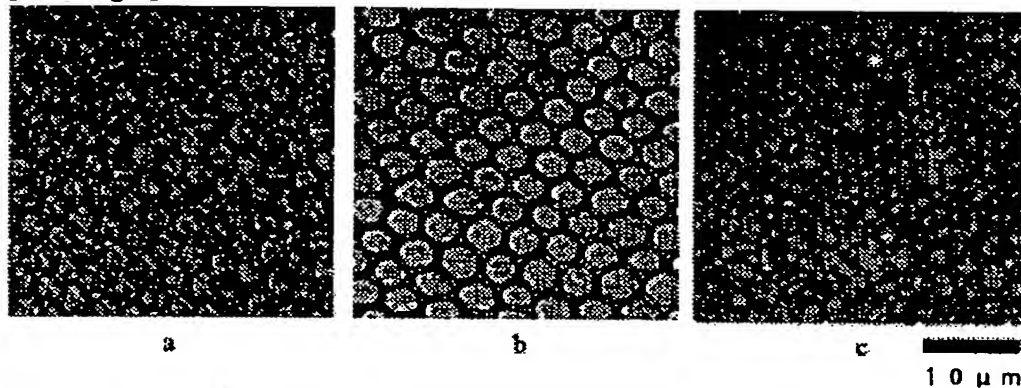


図 1. ポリ乳酸 : C a p a = 1 : 1 , b = 4 : 1 , c = 8 : 1 とした時の  
ハニカム構造体の光学顕微鏡写真

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